

Insight into the Draft Genome Sequence of Human Isolate Lactobacillus rhamnosus LR231, a Bacterium with Probiotic Potential

Padma Ambalam,^a Sheetal Pithva,^b Charmy Kothari,^a Ramesh Kothari,^c Nidhi Parmar,^d Neelam M. Nathani,^d Prakash G. Koringa,^d Chaitanya G. Joshi,^d J. M. Dave,^e B. R. M. Vyas^b

Department of Biotechnology, Christ College, Gujarat, India^a; Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India^b; Department of Microbiology, Christ College, Gujarat, India^c; Department of Animal Biotechnology, Anand Agricultural University, Anand, Gujarat, India^d; Vrindavan Society, Rajkot, India^c

Lactobacillus rhamnosus strain LR231 was isolated from the feces of healthy human subjects. It is observed to be a potential probiotic strain, having a broad spectrum of antimicrobial activity against a wide range of human pathogens and food pathogens. Here, we provide the 2.59-Mb draft genome sequence of L. rhamnosus LR231.

Received 31 January 2014 Accepted 3 February 2014 Published 27 February 2014

Citation Ambalam P, Pithva S, Kothari C, Kothari R, Parmar N, Nathani NM, Koringa PG, Joshi CG, Dave JM, Vyas BRM. 2014. Insight into the draft genome sequence of human isolate *Lactobacillus rhamnosus* LR231, a bacterium with probiotic potential. Genome Announc. 2(1):e00111-14. doi:10.1128/genomeA.00111-14.

Copyright © 2014 Ambalam et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to B. R. M. Vyas, brmvyas@hotmail.com.

The human body constitutes a huge consortium of microbes, the majority of them inhabiting the lower part of the gastro-intestinal (GI) tract. Hence, humans are programmed by their own genome as well as by the environmentally acquired microbiome (1). Various functions of intestinal microbes impact our lives, like food metabolism, antagonist effect on pathogens, and several signaling functions (2, 3). Hence, the intestinal microbiota has a significant influence on various aspects of the host physiology and metabolism, and it is also possible to modulate the genetic composition by changing the environmental conditions, which ultimately leads to a change in the microbial diversity and functions (4).

The consumption of lactic acid bacteria marketed as probiotics is a common approach to maintaining health (5). Currently, probiotics are defined as microorganisms that confer a health benefit on the host when administered in adequate amounts (6). Various minimum criteria have been defined for a strain to be designated a probiotic, such as the microorganism being generally recognized as safe (GRAS), being able to survive the low pH of the GI tract and to adhere to human intestinal cells, being antagonistic to potential pathogens, and having beneficial effects to host (7).

Lactobacillus rhamnosus LR231 is a potential probiotic strain, having a broad spectrum of antimicrobial activity against a wide range of human pathogens and food pathogens. Potential probiotic human strain *L. rhamnosus* LR231 was shown to possess antimicrobial activity against several human pathogens (8).

Whole-genome shotgun sequencing was performed using the 318 Chip and 300-bp chemistry Ion Torrent PGM platform as per the manufacturer's instructions. The draft genome of *L. rhamnosus* LR231 showed the presence of 183 contigs of >200 bp in size when the obtained sequence reads were subjected to reference-guided assembly against the whole-genome sequence of the reference organism *L. rhamnosus* GG using GS Reference Mapper software version 2.3.

The gene annotation and screening for RNAs were performed by submitting the sequences to the Rapid Annotations using Sub-

systems Technology (RAST) server (9). Consequently, 2,607 protein-coding sequences (CDSs) were identified, of which 2,290 CDSs were assigned to one of the 291 RAST subsystems. The genome contains 67 RNA molecules.

The genome analysis showed the strain to possess a relatively high number of CDSs involved in carbohydrate and amino acid metabolism, transport, and virulence-defense mechanisms. The genome contains 40 CDSs encoding about 13 complete phosphoenolpyruvate-carbohydrate phosphotransferase-type transporter systems (PTSs). The organism carries 66 coding sequences related to proteins and enzymes involved in ABC transporters, 7 CDSs coding for antibacterial peptides, and 6 β -lactamases, which thus confirm its broad range of antimicrobial activity and antibiotic resistance properties. Further, this strain exhibits in vitro binding of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and biotransformation, as well as subsequent detoxification and antimutagenic activity (10). Administration of viable LR231 protected rats from MNNG-induced colon inflammation (11). The safety of this strain has been proven in a mouse model (12). Thus, the information obtained from the genome sequence about the various genes involved in the functioning of various metabolic pathways and defense mechanisms will give a better understanding of the antimicrobial activities and probiotic potentials of the strain.

Nucleotide sequence accession number. The sequence of *L. rhamnosus* LR231 has been deposited at GenBank under the accession no. AZHJ00000000.

ACKNOWLEDGMENT

This project was funded by Padma Ambalam.

REFERENCES

 Lahti L, Salonen A, Kekkonen RA, Salojärvi J, Jalanka-Tuovinen J, Palva A, Orešič M, de Vos WM. 2013. Associations between the human intestinal microbiota, *Lactobacillus rhamnosus* GG and serum lipids indicated by integrated analysis of high-throughput profiling data. PeerJ 1:e32. http://dx.doi.org/10.7717/peerj.32.

- 2. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. 2011. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol. 19:349–359. http://dx.doi.org/10.1016/j.tim.2011.05.006.
- Sekirov I, Russell SL, Antunes LC, Finlay BB. 2010. Gut microbiota in health and disease. Physiol. Rev. 90:859–904. http://dx.doi.org/10.1152 /physrev.00045.2009.
- Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. 2008. Highthroughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 57:1605–1605.
- Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. 2005. Probiotic and other functional microbes: from markets to mechanisms. Curr. Opin. Biotechnol. 16:204–211. http://dx.doi.org/10.1016/j.copbio. 2005.02.003.
- Prajapati JB, Nathani NM, Patel AK, Senan S, Joshi CG. 2013. Genomic analysis of dairy starter culture *Streptococcus thermophilus* MTCC 5461. J. Microbiol. Biotechnol. 23:459–466. http://dx.doi.org/10.4014/jmb.1210. 10030.
- Klaenhammer TR, Azcarate-Peril MA, Altermann E, Barrangou R. 2007. Influence of the dairy environment on gene expression and substrate utilization in lactic acid bacteria. J. Nutr. 137:748S-750S.
- Ambalam PS, Prajapati JB, Dave JM, Nair BM, Ljungh Å, Vyas BRM. 2009. Isolation and characterization of antimicrobial proteins produced

- by a potential probiotic strain of human *Lactobacillus rhamnosus* 231 and its effect on selected human pathogens and food spoilage organisms. Microb. Ecol. Health Dis. 21:211–220. http://dx.doi.org/10.3109/089106009 03429052.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.
- Ambalam P, Dave JM, Nair BM, Vyas BR. 2011. In vitro mutagen binding and antimutagenic activity of human Lactobacillus rhamnosus 231. Anaerobe 17:217–222. http://dx.doi.org/10.1016/j.anaerobe.2011.07 .001.
- 11. Gosai V, Ambalam P, Raman M, Kothari CR, Kothari RK, Vyas BR, Sheth NR. 2011. Protective effect of *Lactobacillus rhamnosus* 231 against N-methyl-N'-nitro-N115 nitrosoguanidine in animal model. Gut Microbes 2:319–325. http://dx.doi.org/10.4161/gmic.18755.
- Ambalam P, Ramoliya JM, Dave JM, Vyas BRM. 2013. Safety assessment of potential probiotic *Lactobacillus rhamnosus* 231 and *Lactobacillus rham*nosus V92 in animal model. Int. J. Bioassays 2:333–337.